

Florfenicol Medicated Feed (Aquaflor®) Clinical Field Trials - INAD 10-697

Year 2007 Annual Summary Report on the Use of Florfenicol Medicated Feed in Field Efficacy Trials

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Summary

Florfenicol-medicated feed (Aquaflor®)(FMF) has been used effectively in the U.S. under compassionate INAD Exemption #10-697 to control mortality in a variety of fish caused by common fish bacterial pathogens. In calendar year 2007 the efficacy of FMF was evaluated in 71 disease trials involving approximately 5.4 million fish to control mortality in a variety of test fish caused by a variety of infectious fish pathogens. Trials were conducted at a total of 20 fish culture facilities, including three U.S. Fish and Wildlife Service National Fish Hatcheries (NFH), eight state, seven private, and two tribal fish hatcheries. Use of FMF under Protocol #10-697 allowed the investigator to administer FMF at a dosage of 10 mg/Kg fish/day for 10 days. Overall results indicated that treatment appeared effective in approximately 72% of the trials, ineffective in 8% of the trials, and was characterized as inconclusive in 20% of the trials.

Introduction

The current labels for FMF use in aquaculture limits use to: 1) the control of furunculosis in salmonids caused by *Aeromonas salmonicida*; 2) control of coldwater disease in salmonids caused by *Flavobacterium psychrophilum*; 3) control of enteric septicemia in catfish caused by *Edwardsiella ictaluri*; and 4) control of columnaris in catfish caused by *Flavobacterium columnare*. These label restrictions limit the overall utility of approved FMF use in aquaculture.

Bacterial diseases are a major problem in aquaculture and account for significant losses of fish (Clarke and Scott 1989; Frerichs and Roberts 1989; Bjorndal 1990). Although the importance of environmental conditions (McCarthy and Roberts 1980; Haastein 1988; Munro and Roberts 1989) and the value of effective vaccines, where available (Ellis 1989), are acknowledged, antimicrobial therapy presently has an important role to play in aquaculture (Klontz 1987; Alderman 1988). Florfenicol is a potent, broad-spectrum, antimicrobial agent with bacteriostatic properties (Horsberg et al. 1996). It is a fluorinated analogue of thiamphenicol and is also similar in structure to chloramphenicol, both of which have been used as broad-spectrum, veterinary antibiotics (Nagata and Oka 1996).

Florfenicol has great potential for treatment of infectious diseases, and because of its high potency and safety to humans, it could become an important drug in veterinary medicine, especially with respect to animals used by humans for food

(Powers et al. 1990). Additionally, because florfenicol is not currently used in human medicine, it has become a strong candidate for use in aquaculture, and there is considerable interest to obtain U.S. Food and Drug Administration (FDA) approval for its use in fish culture.

The proposed treatment strategy (i.e., dosage and duration) for the use of FMF in fish is designed to meet the needs of individual fish species, individual fish lots, and a variety of environmental conditions. In all cases, treatment goals are to (1) minimize the negative effects of disease on fish health, quality, and survival, and (2) help meet fishery management objectives. Because many factors can affect the success or failure of florfenicol-medicated feed therapy, supplemental efficacy data from compassionate Investigational New Animal Drug (INAD) use, as well as efficacy data from controlled, replicated studies that are scientifically valid and statistically defensible (i.e., pivotal), are needed to gain approval of FMF use in aquaculture.

Purpose of Report

The purpose of this report is to summarize the results of supplemental FMF field efficacy studies conducted in calendar year 2007 (CY07) . Furthermore, it is expected that these data will be used to enhance the FMF database for the purpose of expanding an appropriate label claim for the use of this new animal drug.

Facilities, Materials, and Treatment Procedures

1. Participating Facilities

A total of 71 effectiveness trials were conducted at 20 fish culture facilities, including three U. S. Fish and Wildlife Service NFH, eight state, seven private, and two tribal fish hatcheries. Trials were conducted to control mortality in a variety of fish caused by a variety of fish pathogens. Water temperature during treatment trials ranged from 33.4 - 82.6 °F, with a mean treatment temperature of 67.9°F.

2. FMF used in trials

The Aquaflor[®] used in CY07 trials contained 500 g of florfenicol per kg of premix. Florfenicol is a pure compound with no inactive ingredients. All florfenicol used was supplied as Aquaflor[®] by Schering-Plough Animal Health, 1095 Morris Avenue, Union, NJ. Florfenicol medicated feed was prepared by either top-coating florfenicol onto commercial fish feed at the Bozeman Fish Technology Center using a Standard Operating Procedure developed by the Service's Aquatic Animal Drug Approval Partnership Program, prepared at the testing site by the Investigator, Monitor, or their designee, or prepared by commercial fish feed manufacturers.

3. Drug dosages and duration

As described in the Study Protocol for INAD #10-697, Investigators were allowed to use FMF at 10 mg of active drug/kg of fish/d for 10 d (approximately 97% of trials were conducted using these treatment regimens).

Study Protocol Deviation: Treatment regimen administered in the remaining trials (approximately 3% of the trials) deviated from the protocol. In one trial involving cabezon, the Investigator noted that the study was terminated on treatment day 6 due to excessive mortality. The Investigator noted that the treatment was started too late on these fish so the entire tank was terminated. In another trial involving tilapia, the Investigator noted that fish had a poor appetite making it difficult to administer the proper amount of active ingredient within a 10 day period. In order to dispense the total amount of drug, feeding a total of 20 days was required. Please note this changed the actual dose from 10 mg of active drug/kg of fish/d to 5 mg of active drug/kg of fish/d. Treated fish will not be available for human consumption for 101 days after the end of the treatment.

Fish Species and Fish Diseases Involved in CY07 Trials

1. Species of fish treated

Eleven fish species, including six species of salmonids, two non-salmonid species, two marine species, and one tropical (aquarium) species were treated

with FMF during CY07. Treated fish ranged in length from 1.6 - 26.0 in. and the mean length of all treated fish was 4.7 in. Fish species treated included:

Salmonids:

brook trout *Salvelinus fontinalis*

brown trout *Salmo trutta*

coho salmon *Oncorhynchus kisutch*

cutthroat trout *O. clarki*

rainbow trout *O. mykiss*

steelhead trout *O. mykiss*

Non-salmonids:

hybrid striped bass *Morone americana* x *M. saxatilis*

tilapia *Oreochromis niloticus*

Marine non-salmonids:

cabazon *Scorpaenichthys marmoratus*

kona kampachi *Seriola rivoliana*

Tropical (Aquarium) Species:

swordtail *Xiphophorus helleri*

2. Diseases treated

The disease treated most frequently was characterized as *Streptococcal septicemia* (51% of the trials). Other diagnosed diseases included: *Aeromonas hydrophila*, bacterial coldwater disease, *Carnobacterium maltaromaticum*, *columnaris*, *Edwardsiella tarda*, furunculosis, motile Aeromonad, saltwater *columnaris*, and *Streptococcus iniae*.

Data Collected

1. Pathology reports

Pathology reports were submitted with 10 trials conducted during CY07. Fish health pathology reports included: 1) a description of how the identity of disease agent(s) was verified; 2) disease identification records that confirm the presence of the disease agent; and 3) the name and title of the individual performing the diagnosis. Additionally, pathology reports often provide documentation that there were no secondary infections or infestations caused by unrelated disease agents in the population of test fish. Pathology reports provide critical information if such submissions are to be used in support of an initial approval, or to expand/extend an existing approved label.

2. Mortality data

As stated in the Study Protocol, mortality data were to be collected 10 days prior to treatment, during the treatment period, and for at least 21 days post-treatment.

Investigators were strongly encouraged to collect mortality data on a daily basis. However, daily collection of pre-treatment mortality data was not always possible due to fish being moved (i.e., split into additional rearing units, or combined with fish from another rearing unit) from rearing unit to rearing unit.

Discussion of Study Results:

1. General observations on the efficacy of FMF for the control of bacterial

diseases in salmonid and non-salmonid fish (Note: Table 1 provides a summary of all efficacious trials; Table 2 provides a summary of all ineffective trials; Table 3 provides a summary of all inconclusive trials; Table 4 provides general CY07 summary data; and Tables 5a and 5b provide a summary of all trials conducted during CY07 under INAD #10-697; (Table 5a is sorted by study number; Table 5b is sorted first by disease treated, second by whether treatments were efficacious or not, and lastly by fish species).

A. Salmonid species - efficacy at 10 mg/Kg fish/d for 10 days under INAD #10-697

Coho salmon, brook, brown, cutthroat, rainbow trout, and steelhead trout, were treated with 10 mg florfenicol/Kg fish/d for 10 days in 27 trials to control mortality caused by *Aeromonas hydrophila*, CWD, *Carnobacterium maltaromaticum*, columnaris, furunculosis, or Motile Aeromonad (Tables 1 - 3). FMF treatments appeared effective in 18 (67%) of the 27 trials; ineffective

in 5 (19%) of the 27 trials; and were characterized as inconclusive in 4 (14%) of the 27 trials.

Please note that approved FMF labels for salmonids diagnosed with furunculosis and CWD became available during CY07. Once these approvals were available, participants were notified that FMF was not to be used under the INAD and needed to be used under the approved label.

B. Non-salmonid species - efficacy at 5 - 10 mg/Kg fish/d for 10 - 20 days under INAD #10-697

Hybrid striped bass and tilapia were treated with 5 - 10 mg florfenicol/Kg fish/d for 10 - 20 days in 40 trials to control mortality caused by *Streptococcal septicemia*, *Streptococcus iniae*, or *Edwardsiella tarda* (Tables 1 & 3). FMF treatments appeared effective in 31 (78%) of the 40 trials and were characterized as inconclusive in 9 (22%) of the 40 trials.

C. Marine non-salmonid species - efficacy at 10 mg/Kg fish/d for 6 - 10 days under INAD #10-697

Cabezon and kona kampachi were treated with 10 mg florfenicol/Kg fish/d for 6 - 10 days in three trials to control mortality caused by saltwater columnaris or *Streptococcus iniae* (Tables 1 & 2). FMF treatments appeared effective in 2 (67%) of the three trials and was ineffective in one (33%) of the three trials.

D. Tropical (Aquarium) species - efficacy at 10 mg/Kg fish/d for 10 days under INAD #10-697

Swordtail were treated with 10 mg florfenicol/Kg fish/d for 10 days in one trial to control mortality caused by columnaris (Table 3). FMF treatment was characterized as inconclusive in this trial.

2. Observed Toxicity

No toxicity or adverse effects relating to FMF treatment were reported.

Number of Treated Fish under Slaughter Authorization

Total number of treated fish during CY07 was 5,398,955. The total number of treated fish to count against the slaughter authorization dated November 15, 2005 (valid through September 16, 2007) is 30,850,886. The total number of treated fish to count against the current slaughter authorization dated September 17, 2007 is 657,659. No changes have occurred to the current FLOR (Aquaflor®) INAD #10-697 study protocol.

Facility Sign-up List

Please see “Table 6. Facilities and Names of Investigators” for facilities that signed-up to participate in the FLOR (Aquaflor®) INAD #10-697 during CY07.

Facilities not listed in the current FLOR (Aquaflor®) INAD #10-697 study protocol have been highlighted.

Summary of Study Results

Florfenicol medicated feed (Aquaflor®) was administered to test fish in 71 separate trials at a dosage of either 5 or 10 mg/Kg fish/d for 6 - 20 d. Eleven different fish species were treated with FMF, and trials involved approximately 5.4 million fish. Treated fish ranged in size from 1.6 - 26 in. Water temperature during treatment ranged from 33.4 - 82.6 °F, with a mean treatment temperature of 67.9 °F. Overall results showed that in approximately 72% of the trials, FMF treatments appeared effective, 8% of the trials were ineffective, and 20% of the trials were characterized as inconclusive. Although data from these trials will be considered ancillary, trial results should provide useful corroborative data to support a new/expanded label claims for FMF. It is anticipated that additional ancillary efficacy data will continue to be collected under INAD #10-697. In future trials conducted under INAD #10-697, efforts will continue to be directed towards the generation of high quality data.

References

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Table 1. Summary of CY07 Florfenicol Medicated Feed (Aquaflor®) Efficacy Results - Effective Trials

Hatchery	Number of Trials	Fish Species	Fish Size (inches)	Number of Fish	Disease	Number of Treatment Days	Dose (mg/kg)	Temp. (°F)
Silver Moon Springs	2	BKT	4.0 - 4.5	490,000	Furunculosis	10	10	61.0 - 64.0
Watersmeet Trout Hatchery	1	BKT	2.70	25,000	<i>A. hydrophila</i>	10	10	44.0
Eagle Fish Health Lab	1	BNT	3.75	39,675	Furunculosis	10	10	60.5
Oden SFH	1	BNT	6.90	3,730	<i>A. hydrophila</i>	10	10	45.7
	3	BNT	16.9 - 21.1	4,706	<i>Carnobacterium maltaromaticum</i>	10	10	45.3
Silver Moon Springs	1	BNT	10.00	24,000	Furunculosis	10	10	64.0
The Abalone Farm	1	CAB	4.00	650	Saltwater Columnaris	10	10	54.0
Makah NFH	1	COS	3.80	257,335	Furunculosis	10	10	60.9
Nez Perce Tribal Hatchery	1	COS	5.00	293,211	CWD	10	10	41.0
Prosser Hatchery	1	COS	4.30	66,000	CWD	10	10	35.8
Murray Springs Trout SFH	1	CUT	3.03	19,318	CWD	10	10	52.0
Kona Blue Water Farms	1	KON	9.50	44,000	<i>Streptococcus iniae</i>	10	10	70.0
Fish Barn	1	NTI	10.20	9,150	<i>Edwardsiella tarda</i>	10	10	80.5
	2	NTI	11.40	53,453	<i>Streptococcus iniae</i>	10	10	77.5
Eagle Fish Health Lab	1	RBT	10.00	39,000	Furunculosis	10	10	62.5

Table 1. Summary of CY07 Florfenicol Medicated Feed (Aquaflor®) Efficacy Results - Effective Trials - cont.

Hatchery	Number of Trials	Fish Species	Fish Size (inches)	Number of Fish	Disease	Number of Treatment Days	Dose (mg/kg)	Temp. (°F)
Klamath SFH	1	RBT	2.25	1,000,000	CWD	10	10	49.0
Loa SFH	1	RBT	3.10	59,857	Columnaris & CWD	10	10	59.0
Watersmeet Trout Hatchery	1	RBT	2.20	20,000	<i>A. hydrophila</i>	10	10	44.0
White Sulphur Springs NFH	1	RBT	3.50	91,536	CWD	10	10	48.0
Kent SeaTech Corp	27	SXW	1.6 - 6.4	1,775,789	<i>Streptococcal septicemia</i>	10	10	79.0
Fish Barn	1	NTI	10.60	4,085	<i>Edwardsiella tarda</i>	20	5	82.6

Table 2. Summary of CY07 Florfenicol Medicated Feed Efficacy (Aquaflor®) Results - Ineffective Trials

Hatchery	Number of Trials	Fish Species	Fish Size (inches)	Number of Fish	Disease	Number of Treatment Days	Dose (mg/kg)	Temp. (°F)
The Abalone Farm	1	CAB	3.00	780	Saltwater Columnaris	6	10	54.0
Murray Springs Trout SFH	2	CUT	3.1 - 3.5	45,974	CWD	10	10	52.0
Harrietta Hills Trout Farm	1	RBT	11.90	212,299	Columnaris	10	10	49.0
Coleman NFH	2	STT	2.7 - 3.0	3,240	Columnaris	10	10	65.3

Table 3. Summary of CY07 Florfenicol Medicated Feed (Aquaflor®) Efficacy Results - Inconclusive Trials

Hatchery	Number of Trials	Fish Species	Fish Size (inches)	Number of Fish	Disease	Number of Treatment Days	Dose (mg/kg)	Temp. (°F)
Marquette SFH	2	BKT	2.6 - 3.4	134,000	Motile Aeromonad	10	10	46.0
Crystal Lake Hatchery	1	COS	3.70	151,037	CWD	10	10	33.4
Eagle Fish Health Lab	1	RBT	7.94	130,000	Furunculosis	10	10	62.5
Tropical Aquaculture Lab	1	SWT	2.10	75	Columnaris	10	10	79.0
Kent SeaTech Corp	9	SXW	1.6 - 6.4	401,055	<i>Streptococcal septicemia</i>	10	10	79.0

Table 4. Summary Data Regarding CY07 Florfenicol Medicated Feed (Aquaflor®) Efficacy Trials

Total Fish Treated:	<u>5,398,955</u>
Number of fish treated in effective trials	4,320,495
Number of fish treated in ineffective trials	262,293
Number of fish treated in inconclusive trials	816,167
Total number of trials:	71
Number of effective trials:	51
Number of ineffective trials:	6
Number of inconclusive trials:	14
Treatment Regimes Used:	
10 mg/Kg fish/day for 10 days	69 trials
10 mg/Kg fish/day for 6 days	1 trial
5 mg/Kg fish/day for 20 days	1 trial
Treatment Water Temperature (°F):	
Temperature Range	33.4 - 82.6
Mean Temperature	67.9
Size of Treated Fish (in.):	
Size Range	1.60 - 26.0
Species Treated:	
<u>Salmonids:</u>	
brook trout <i>Salvelinus fontinalis</i>	
brown trout <i>Salmo trutta</i>	
coho salmon <i>Oncorhynchus kisutch</i>	
cutthroat trout <i>O. clarki</i>	
rainbow trout <i>O. mykiss</i>	
steelhead trout <i>O. mykiss</i>	

Non-salmonids:

hybrid striped bass *Morone americana* x *M. saxatilis*
tilapia *Oreochromis niloticus*

Marine non-salmonids:

cabezon *Scorpaenichthys marmoratus*
kona kampachi *Seriola rivoliana*

Tropical (Aquarium) Species:

swordtail *Xiphophorus helleri*